Analysis of NMR Spin-lattice Relaxation Dispersion on Complex Systems

Yang Huang
Front cover: OEC complex of PSII, NMRD profiles and Liouville equation.

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Analys av NMR spinn-lattice relaxations dispersion på komplexa system

Yang Huang
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Veritati et sapientiae semper studeamus.

吾恒求真理与智慧。
To my spiritual fathers:

Carl Philipp Gottfried von Clausewitz
(1780-1831)

&

Johann Sebastian Bach
(1685-1750)

and to my supervisor, tutor:

Prof. Per-Olof Westlund

&

Dr. Tobias Sparrman

Without these people this thesis wouldn’t have been carried out.
‘...A powerful emotion must stimulate the great ability of a military leader, whether it be ambition as in Caesar, hatred of the enemy as in Hannibal, or the pride in a glorious defeat, as in Frederick the Great.

Open your heart to such emotion. Be audacious and cunning in your plans, firm and persevering in their execution, determined to find a glorious end, and fate will crown your youthful brow with a shining glory, which is the ornament of princes, and engrave your image in the hearts of your last descendants.’

C. von Clausewitz: Principles of War (1812)
王静安
人间词话

古今之成大事者，大学问者也。经过三境界，

昨夜西风吹白日，碧阶携酒上高楼，望尽天涯路。

第一境也：衣带渐宽终不悔，为伊消得人憔悴。此第二境也。

第二境也：众里寻他千百度，回头那人却在灯火阑珊处。此第三境也。

第三境也：众里寻他千百度，蓦然回首那人却在灯火阑珊处。
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Abstract

This thesis focuses on the analysis of spin-lattice NMRD relaxation profiles measured in various complex systems such as proteins, zeolites and ionic liquids. Proton, deuterium and fluoride T1-NMRD relaxation profiles were obtained from a fast-field cycling (FFC) instrument. It is found that it is also possible to obtain NMRD profiles from the molecular dynamics (MD) simulation trajectories. NMRD Profiles were analyzed by using different relaxation models, such as the Solomon-Bloembergen-Morgan (SBM) theory and the Stochastic Liouville (SL) theory.

Paper I described the hydration of protein PrxV obtained from a MD simulation, and compared with the picture emerges from an analysis by using a generally accepted relaxation model [appendix C]. The result shows that the information from NMRD analysis is an averaged picture of water molecules with similar relaxation times; and the MD simulations contains information of all types of interested water molecules with different residence times.

In paper II NMRD profiles have been used to characterize the hydration of the oxygen-evolving complex in state S1 of photosystem II. NMRD experiments were performed on both intact protein samples and Mn-depleted samples, and characteristic dispersion differences were found between 0.03 MHz to 1 MHz; approximately. Both the SBM theory and the SL theory have been used to explain this dispersion difference, and it is found that this is due to a paramagnetic enhancement of 1-2 water molecules nearby ~10 Å from the spin center of the Mn4CaO5 cluster. The result shows the reorientation of the molecular cluster is in μs time interval. When compare these two theories, the SL theory presented a better interpretation because parameters obtained from the SBM theory shows they didn’t fulfil the presupposed perturbation criterion (the Kubo term).

Paper III deals with the water dynamics in the restricted/confined spaces in the zeolite samples (H-ZSM-5 and NH4-ZSM-5) and obtained by proton and deuterium spin-lattice NMRD profiles. The results show that the spin-lattice NMRD can be used to characterize various zeolites. The temperature has a weak effect on the relaxation rate R1, but the change of different counter ions may change the hydration and the translational diffusion pores and give different R1.

Proton and fluoride NMRD profiles and MD simulations were both used to study the dynamics of BMIM[PF6] in paper IV. Results indicate the
reorientation of the molecules are in the ns time regime, and the effective correlation time obtained from $^1$H and $^{19}$F are the same. From the MD simulation it is found the reorientation of $[PF_6]$ ions is much faster (in ps) compare with BMIM$^+$ ion which moves in the ns time range.

With previous results, the FFC NMRD profiles are indeed very informative tools to study the molecular dynamics of complex systems. The MD simulation can be used as a complementary method to obtain detailed information. By combine these two methods, it provide a more colorful picture in the study of protein hydration and liquid molecular dynamics.

**Keywords:** NMRD, relaxation theory, hydration, MD, FFC, PrxV, OEC complex, ZSM-5, BMIM [PF$_6$]
Abstract

Avhandlingen fokuserar på analyser av spinn-lattice ($T_1$) NMRD relaxationsprofiler som uppmätts på olika system som t.ex. proteiner, zeoliter och joniska vätskor. Proton, deuterium och fluorid $T_1$-NMRD relaxationsprofiler har erhållits från ett field cycling instrument. Vi har även beräknat $T_1$-NMRD profiler med hjälp molekylära dynamiken (MD) trajectorer. NMRD profilerna analyserades genom att använda olika relaxationsmodeller typ Solomon-Bloembergen-Morgan (SBM) teori och stokastisk Liouville (SL) teorin.

I artikel I beskrivs hydratisering av protein PrxV med hjälp av en MD simulering och den jämfördes därefter med den bild som kommer ur en analys gjord med en allmänt accepterad relaxationsmodell [appendix C]. Resultatet visar att informationen från NMRD profilen är en medelvärdes bild över olika vattenmolekyler med liknande uppehållstider medan MD simuleringar innehåller information om alla typer av vattenmolekyler med olika uppehållstider.

I artikel II har proton $T_1$ NMRD profiler använts för att karakterisera hydratiseringen av fotosystems II:s syre genererande molekylkomplex i S1 tillståndet. NMRD experimenten utfördes på både intakta proteinprov och där Mn- tagits bort. En karakteristisk skillnad mellan NMRD profilerna konstaterades i frekvensintervallet 0.03 MHz till 1 MHz. Både SBM teorin och SL teorin har använts för att förklara denna dispersion skillnad och det visar sig att detta beror på en paramagnetisk relaxationsökning av 1-2 vattenmolekyler i närheten, ca 10 Å, från spinn centrum hos Mn$_4$CaO$_5$ klastret. Resultatet visar på att omorienteringen av molekylklastret är i us tidsintervallet. När dessa två teorier jämförs ger SL teorin en mer realistisk tolkning eftersom parametrarna som erhölls från SBM teorin visar att de inte uppfyller giltighets kriteriet (Kubo termen).

Artikel III handlar om vattendynamik i begränsade/trånga utrymmen i zeolitproven H-ZSM-5 och NH$_4$-ZSM-5 och som erhållits från proton och deuterium spin-lattice NMRD profiler. Resultatet visar att spinn-lattice NMRD kan användas för att karakterisera olika zeoliter. Temperaturen har en svag inverkan på relaxationshastigheten $R_\text{R}$, men bytet av olika motjoner kan ändra hydratisering och translations diffusion i porerna och ger olika $R_\text{R}$.

I artikel IV användes både proton och fluor NMRD profiler samt MD-simuleringar för att studera dynamiken i BMIM[PF$_6$]. Resultaten tyder på att omorientering av molekylerna är i ns och den effektiva korrelationstiden
erhålls från \(^1\)H och \(^{19}\)F är densamma. Från MD simulering får vi omorienteringen av [PF\(_6\)]\(^-\) jonen vilken är mycket snabbare (ps) jämfört med BMIM\(^+\) jonen vilken rör sig i ns tidsintervallet.

Som även tidigare resultat visar är FFC NMRD profilerna verkligen mycket informativa för att studera molekyl dynamiken i komplexa system. MD simulering kan användas som en kompletterande metod för att erhålla detaljerad information. Genom att kombinera dessa två metoder ger en mer nyanserad bild av vatten i protein och vätskemolekulernas dynamik.

**Keywords:** NMRD, avkoppling teori, hydrering, MD, FFC, PrxV, OEC komplex, zeolit ZSM-5, BMIM [PF\(_6\)]
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>$B_0$</td>
<td>magnetic field</td>
</tr>
<tr>
<td>BPOL</td>
<td>polarisation field (MHz)</td>
</tr>
<tr>
<td>BRLX</td>
<td>relaxation field (MHz)</td>
</tr>
<tr>
<td>°C</td>
<td>degree Celsius</td>
</tr>
<tr>
<td>$\Delta zfs$</td>
<td>transient ZFS (1/cm)</td>
</tr>
<tr>
<td>$\eta$</td>
<td>asymmetry parameter</td>
</tr>
<tr>
<td>FID</td>
<td>free induction decay</td>
</tr>
<tr>
<td>FFC</td>
<td>fast-field cycling</td>
</tr>
<tr>
<td>$g$</td>
<td>g-factor</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>gyromagnetic ratio (or magnetogyric ratio)</td>
</tr>
<tr>
<td>$\gamma(t)$</td>
<td>a stochastic function</td>
</tr>
<tr>
<td>$h$</td>
<td>Planck constant ($h=6.62620\times10^{-34}$ JS)</td>
</tr>
<tr>
<td>$\hbar$</td>
<td>Planck constant ($\hbar = h/2\pi$)</td>
</tr>
<tr>
<td>$\hbar^{DD}(t)$</td>
<td>stochastic quantity</td>
</tr>
<tr>
<td>IL</td>
<td>ionic liquid</td>
</tr>
<tr>
<td>$J(\omega)$</td>
<td>spectral density</td>
</tr>
<tr>
<td>K</td>
<td>Kelvin ([K] = [°C] + 273.15)</td>
</tr>
<tr>
<td>$\chi$</td>
<td>quadrupole coupling constant</td>
</tr>
<tr>
<td>$L(t)$</td>
<td>single Lorentzian function</td>
</tr>
<tr>
<td>M</td>
<td>magnetic moment</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>MD</td>
<td>molecular dynamic</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>$M_x, M_y, M_z$</td>
<td>components of the magnetization vector</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>NMRD</td>
<td>nuclear magnetic resonance dispersion</td>
</tr>
<tr>
<td>$\mu_0$</td>
<td>magnetic field constant</td>
</tr>
<tr>
<td>$\nu$</td>
<td>microwave frequency (in Hz)</td>
</tr>
<tr>
<td>OEC</td>
<td>oxygen-evolving complex</td>
</tr>
<tr>
<td>$\omega$</td>
<td>Larmor frequency</td>
</tr>
<tr>
<td>PRE</td>
<td>paramagnetic enhanced relaxation</td>
</tr>
<tr>
<td>PrxV</td>
<td>Peroxiredoxin 5</td>
</tr>
<tr>
<td>$R_1$</td>
<td>relaxation rate</td>
</tr>
<tr>
<td>$r_{i,x}$</td>
<td>distance between the nuclear spin and the x spin</td>
</tr>
<tr>
<td>SBM</td>
<td>Solomon-Bloembergen-Morgen theory</td>
</tr>
<tr>
<td>SL</td>
<td>slow-motion theory</td>
</tr>
<tr>
<td>$S_{ix}$</td>
<td>spectral density function, $i = 1, 2, 3$</td>
</tr>
<tr>
<td>$S_T$</td>
<td>spin quantum number</td>
</tr>
<tr>
<td>SWT</td>
<td>switching time</td>
</tr>
<tr>
<td>$T_{1,x}$</td>
<td>spin-lattice relaxation (or longitudinal relaxation)</td>
</tr>
<tr>
<td>$T_{2,x}$</td>
<td>spin-spin relaxation (or transverse relaxation)</td>
</tr>
<tr>
<td>TCF</td>
<td>time correlation function ($C(\tau)$)</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>---------------------</td>
</tr>
<tr>
<td>$\tau_c^x$</td>
<td>effective correlation time</td>
</tr>
<tr>
<td>$\tau_R$</td>
<td>reorientation correlation time</td>
</tr>
<tr>
<td>$\tau_{ZFS}$</td>
<td>correlation time</td>
</tr>
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List of Publications

This thesis is based on the following papers and manuscripts which are referred to in the text by the corresponding Roman numerals.

I. **Yang Huang**, Kwangbo Nam, Per-olof Westlund*
   The water R1(ω) NMRD profiles of a hydrated protein from molecular dynamics simulation
   Physical Chemistry, Chemical Physics - PCCP, ISSN 1463-9076, E-ISSN 1463-9084, Vol. 15, no 33, 14089-14097, 2013.

II. Guangye Han, **Yang Huang**, Faisal.H.M Koua, Jian-Ren Shen, Per-olof Westlund* Johannes Messinger*
    Hydration of the oxygen-evolving complex of photosystem II probed in the dark-stable S, state using proton NMR dispersion profiles

III. **Yang Huang**, William Siljebo and Per-Olof Westlund*
    Water Proton and Deuterium spin-lattice relaxation in Zeolite ZSM-5 by Fast Field-Cycling NMR relaxometry
    2015, in manuscript.

IV. **Yang Huang**, Tobias Sparrman, Y-L.Wang, A.Laaksonen, and Per-olof Westlund*
    Analysis of Proton/Fluoride Spin-Lattice NMR Dispersion Experiment of an Ionic Liquid, BMIM[PF6] by using Molecular Dynamic Simulations and Relaxation Theory
    2015, in manuscript

All papers have been reprinted with kind permission from the publishers.

Work Contribution:

I: YH performed all the NMRD simulations of MD-data and analysis of NMRD profiles.
II: YH performed all the theoretical numerical analysis of NMRD profiles.
III: YH performed all the theoretical numerical analysis of NMRD profiles.
IV: YH performed all the theoretical numerical simulation and analysis of MD-data and NMRD profiles.
The aim of this Ph.D. thesis is to analyses the spin-lattice NMRD (nuclear magnetic relaxation dispersion) relaxation (T$_1$-NMRD relaxation) results from experimental and simulation work. Four projects have been carried out and will be discussed in the thesis:

1. Analyze of proton T$_1$-NMRD profiles in a hydrated protein using MD (molecular dynamics) simulations.
2. Analyze of proton T$_1$-NMRD profiles in confined space (oxygen evolving complex, OEC) from experimental results.
3. Analyze of proton and deuterium (^2H) T$_1$-NMRD profiles experiments on zeolite ZSM-5.
4. Analyze of proton and fluoride (^19F) T$_1$-NMRD profiles experiments on ionic liquid BMIM[PF$_6$]; both NMRD and MD simulation.

The thesis will firstly start with a brief introduction and some basic concepts of the relaxation theory. And then all four papers will be summarized as follow. Finally a conclusion section will also be given.

* This thesis is the extend work followed my master thesis ("Theoretical and experimental study of water proton relaxation in liquid, ice and the effect of paramagnetic Gd$^{3+}$($H_2O)_8$ complex.") in the study of relaxation theory. By that thesis, I've already given basic introduction of NMR, and detailed explanation of theoretical methods in density matrix, equation of motion, the pure dephasing relaxation model and the dipolar interactions of spin like system. Therefore I'll not repeat it here again.

* Master thesis can be found at:

1. Introduction

1.1 General background of NMRD

The thesis start with a short introduction of NMR, then focus on describing the meaning of spin-lattice NMR Dispersion relaxation, and why one should analyse it.

Nuclear magnetic resonance (NMR) is a powerful method which is commonly applied in biology, chemistry, material science, medicine and physics as well to study the molecule structure and dynamics of systems. It is a popular technique such as MRI (magnetic resonance imaging) and has already been well known and used for several decades. Multiple awards are given to related topics and especially in year 1991 and 2002 when the Nobel prizes in chemistry was awarded to R.Ernst and K.Wüthrich for their development of NMR techniques [1.1]. The most common use of NMR is for determine the structure of molecules.

The abbreviation NMRD stands for ‘nuclear magnetic relaxation dispersion’. The word ‘dispersion’ here can be described as the rapid reduction of the longitudinal relaxation rate R. [1.2]. Alternatively it is the frequency dependence of R. which contains dynamical information of X-solvent interactions (e.g. protein-solvent interaction) [1.3-1.7].

The earliest publication using this term is in 1969, carried out by S.Koenig et al [1.3] with a study in protein solution for Apotransferrin; followed a very pioneer study of the relaxation effects in the nuclear magnetic resonance absorption by N.Bloembergen et al., [1.4] in 1948. And now it is almost 50 years since the very first draft of proton relaxation studies of water-protein interactions and dynamics. [1.5]

The NMRD profiles/disersion curve is measured with a FFC (fast field-cycling) instrument, details of FFC can be seen in chapter 2. Dispersion is the frequency dependence of the relaxation rate, and eventually there are two types of relaxation: spin-lattice/longitudinal relaxation (notation: T₁, where T stands for relaxation time constant), and spin-spin/transverse relaxation (notation: T₂). This work will focus on longitudinal relaxation.
As an example, fig.1.1. shows proton T\(_1\)-NMRD profiles of a protein at three different temperatures, and it displays the proton longitudinal relaxation rate R\(_1\) (R\(_1\) is defined as 1/T\(_1\)) versus the Larmor frequency. In NMRD studies the probed proton Larmor frequencies typically ranges from 10 kHz to 40 MHz. The magnetic field is generated by an electromagnet. A conventional NMR spectrometer provides relaxation times at higher frequencies in a single measurement; e.g. in paper III it used 360 MHz.

When working with the NMRD method, it doesn't have to be the proton Larmor frequency (\(\omega\)), other nuclei such as deuterium/\(^2\)H, carbon/\(^{13}\)C, fluorine/\(^{19}\)F, phosphorus/\(^{31}\)P, etc. are also possible [1.8-1.10]. The present research have studied proton, deuterium and fluorine NMRD profiles. The Larmor frequency \(\omega\) is defined by \(\omega = \gamma B_0\) where \(\gamma\) stands for the gyromagnetic ratio, and it's the ratio of magnetic dipole moment to its angular momentum; B\(_0\) is the magnetic field. Different nuclei have different \(\gamma\) values and thus the Larmor frequency can be calculated, respectively.

The NMRD profiles provide information from reorientation dynamics of the solvent. In a system, the solvent interact with macromolecular surfaces
which locally affect the solvent reorientation. And by chemical exchange the bulk solvent carries this information about the surface interaction. Thus proton R\textsubscript{1}-NMRD profiles is very commonly used in protein studies because water often plays an important role in studies of structure and dynamics of biological systems.

The experimental proton relaxation rate contains information about the time modulation of the dipole-dipole interaction for protons. The steepest descent of dispersion indicates the timescale of dipole-dipole fluctuations; e.g. at about 200 kHz in fig.1.1. The characteristic correlation time \( \tau_c \) can be extracted from the time correlation function over a selected frequency regime. In detail molecular dynamics are contained in the spectral density function which could be extracted from the NMRD profiles. This part will be discussed later with detail in chapter 2.

The longitudinal relaxation rate R\textsubscript{1} is the measurement over the solvent (e.g. water) and indeed it measure the average dynamical properties. For example one cannot point out individual dynamical information of a selected macromolecule.

The mechanism of relaxation includes dipole-dipole (DD) interaction, quadrupole interaction, spin rotation interaction, chemical shift anisotropy, etc. Details of dipole-dipole and quadrupole interactions were discussed in the papers.

This chapter we have only given a brief introduction in general and more details are formed in chapter 2. For those who are interested in relaxation theory as a research topic related books are given in reference [1.2], and [1.11] - [1.16]. Here [1.2], [1.11] and [1.12] are fundamental books, and advanced readers may go to [1.13] - [1.16] directly.
1.2 Topics that are covered in the thesis (based on publications):

**In paper I**, to distinguish the characteristics of different types of water-biomolecule interaction from NMRD profiles would be rather difficult by using traditional experimental methods. By means of MD simulations we questioned whether it is possible to unravel the problem. In this paper, we use the MD simulation trajectories as a main source to calculate the water proton T₁-NMRD profiles which are called “exact” NMRD profiles (fig 5, paper I). Those “exact” profiles are interpreted by using a generally accepted simply model by K.Venu et al., [1.17].

**In paper II**, what happens after the elimination of manganese from the OEC complex of PSII? Does the protein structure get changed? The paper intended to investigate the difference in characteristic dispersion between experimental results of two different samples. We hypothesized that it is due to a paramagnetic enhanced relaxation (PRE) or by water protons, whereas not from structural changes caused by the elimination Mn.

**In paper III**, what pore sizes do ZSM-5 exhibit? What information about ZSM-5 can be obtained from ¹H and ²H NMRD profiles? As shown in the results, there is an intensive increase of the relaxation rate R₁ in the high field regime; but why it is not presented for ²H FFC experiments? Are there any temperature dependences of the spin-lattice relaxation rate for ZSM-5? We try to cover these questions in this paper.

**In paper IV**, by applying MD together with proton and fluorine T₁-NMRD experiments we can investigate the dynamics of BMIM⁺, [PF₆]⁻ and BMIM⁺[PF₆]⁻ ions. Due to the long-range electrostatic interactions in ILs strong correlation between ionic groups are expected [1.18]. And we therefore ask the question whether MD and NMRD methods can detect these correlations? What is then the time range of the molecular reorientational dynamics of BMIM[PF₆]⁻?
2. Methods

In order to study the four presented complex systems from chapter 1, theoretical/computational models were used for analyse. The main idea is to find a theoretical interpretation of theory and experiments. For example, in paper II experiments with intact and Mn-depleted OEC samples were performed. The difference between two results, the relaxation enhancement is described by the traditional Solomon-Bloembergen-Morgen (SBM) theory [2.1-2.3] and the Stochastic Liouville theory based on slow-motion (SL) theory [2.4-2.6].

Fig.2.1. Displays a flow chart of the main theme: How theoretical and experimental results (both empirical and simulation) are ‘glued’ together by analysis.

This chapter starts by introducing relaxation, theory, MD, NMRD experiments and ends up with the analysis as presented in fig.2.1. It will not focus on the derivation of equations but rather to give all needed core information for readers to understand the summary and conclusion sections. Full description can be found in reference literatures suggested in chapter 1.
2.1 Relaxation theory

Relaxation

Consider a system is perturbed from its thermodynamic equilibrium and turns to a nonequilibrium state; the system will returns to equilibrium spontaneously after a certain period of time. This process of returning to its equilibrium is named relaxation. [1.2]

Spin

The property spin is a central in NMRD dispersion, NMR and MRI. Spin is one of four characteristic properties of atomic nuclei; the others are mass, magnetism, and electric charge [2.7]. It is a quantum-mechanical intrinsic property of a particle, and in a classical picture it is approximately describes as an object rotating and in space.

Historically the study of spin can traced back to year 1896 when Zeeman performed an interesting experiment with sodium, and observed the splitting of spectral lines that lead to the discovery of the ‘Zeeman splitting’; and he was rewarded the Nobel Prize in 1902 together with Lorentz [1.1]. Later in 1928 Dirac provided quantum mechanics based equations to explain more details about the spin [2.8-9].

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Fig.2.1.1 An illustrative display shows the splitting in the spectral lines from Zeeman’s experiments.
Spin is not only presented for the electron but a general property for many particles. There are two types: half-integer spin (such as proton, electron, positron, and etc.) and integer spin (e.g. photon). And there are only certain number of possible spin states for a particle with certain spin number. Nuclei such as $^1$H, $^{13}$C, $^{15}$N and $^{31}$P have the spin number $\frac{1}{2}$. In this case they show two available states $+\frac{1}{2}$ and $-\frac{1}{2}$ (fig.2.1.2.); whereas $^2$H has spin number equal to 1, with three states 1, 0 and -1 [2.10].

**Spin Relaxation**

For a spin system without any external regulation/no added external magnetic fields; when applied this external magnetic field to the spin system it will change the spin state populations.

![Spin States](image)

Fig.2.1.2. The figure shows the energy levels of spin $\frac{1}{2}$. Notice that the up (↑) state has lower energy. $B_0$ is the static magnetic field.

Consider this simple case with spin number $\frac{1}{2}$ (fig.2.1.2), for which there are only two different spin states (for better imagination one could think about up and down arrows): up (↑) / $\alpha$ state and down (↓) / $\beta$ state. $N_{\text{upper}}$ and $N_{\text{lower}}$ are the population of spins in different states after the system reaches equilibrium [2.11].

The equilibrium Boltzmann distribution of spin population is given by:

\[
\frac{N_{\text{upper}}}{N_{\text{lower}}} = \exp\left(-\frac{\Delta E}{kT}\right) = \exp\left(-\frac{\hbar \omega}{kT}\right)
\]

(2.1)
Here $T$ is the absolute temperature of the spin system, and $k$ is the Boltzmann constant $= 1.38 \times 10^{-23}$ J/K; $h$ is the Planck constant $= 6.63 \times 10^{-34}$ J$\cdot$s, $\nu$ is the frequency, and $\Delta E$ is the energy difference between two states.

For a magnetic field of 1 T the spin population difference calculated by equation (2.1) from one million proton spins at room temperature will be about 7 spins per million contributing to the net magnetization of the NMR signal. The reader can calculate the value ‘7’ by using equation (2.1), which temperature $T = 293$ K, and frequency $\nu = 42$ MHz =1 T. A net magnetic moment in the spin system is caused by the difference between $N_{\text{upper}}$ and $N_{\text{lower}}$.

![Fig.2.1.3](image)

Fig.2.1.3. Figure shows the spin populations of different spin states before (a) and after (b) an extra magnetic field (RF/radio frequency pulse, $B_{\text{extra}}$) is added perpendicular to $B_0$. The magnetization vector is shown from the view of $Z$ direction (the longitudinal direction). The nucleus at lower energy state will transition to the higher state by absorbing energy. (NMRD switches $B_0$ to follow $T_1$ relaxation between new energy states, and finally an RF pulse is used to read out the population difference.)

After the presented equilibrium system is perturbed by and extra magnetic field, a new equilibrium will be created (see fig.2.1.3,b). Then remove $B_{\text{extra}}$ spins will intend to recover to its equilibrium population distribution again and in NMR this process is named as **spin-lattice relaxation**.

As mentioned in chapter 1 the two groups (spin-lattice and spin-spin) of relaxation process differ by its way of energy exchange between a nucleus and the environment: spin-lattice relaxation restores the spin population, and spin-spin relaxation indicate the loss of coherence between different spin states.
**Bloch equations**

The most pioneer explanation of spin relaxation was carried out by Bloch equations in 1946 [2.12]. The phenomenological relaxation time $T_1$ and $T_2$ are given by:

\[
\frac{dM_x}{dt} = \frac{M_0 - M_x}{T_1}
\] (2.2)

\[
\frac{dM_y}{dt} = M_y \omega - \frac{M_y}{T_2}
\] (2.3)

\[
\frac{dM_z}{dt} = -M_z \omega - \frac{M_z}{T_2}
\] (2.4)

In equations (2.2) to (2.4), $\omega$ is the Larmor frequency after the name who found it, and it equals $\gamma B_0$ which $\gamma$ is gyromagnetic ratio. The unit of frequency is in Hz or in rad s$^{-1}$; in FFC we always use MHz. $M_x$, $M_y$, and $M_z$ are magnetization components in three directions where $M_0$ is the value at equilibrium; $T_1$ and $T_2$ are two types of relaxation time mentioned in chapter 1. Equation (1) describe the time-dependence of the Z component/along the external field of the magnetization vector; which the inverse of $T_1$ denote the rate of $M_z$ return to equilibrium.

In math we have simple solution for such kind of function:

\[
\frac{dx(t)}{dt} = \frac{a - x(t)}{b}
\]

\[
x(t) = a + [\text{cons}] \exp(-t / b)
\] (2.5)

; and the solution to equation 2.2 is:

\[
M_z(t) = M_0 + [\text{cons}] \exp(-t / T_1)
\] (2.6)

Bloch equations are only valid for uncoupled spins and as it is phenomenological, which means they cannot draw any quantitative conclusion on the physical explanation of relaxation processes.
Spin-lattice relaxation time $T_1$

The spin-lattice/longitudinal relaxation time $T_1$ is the rate of energy transfer from the nuclear spins to lattice; where the word lattice refers to the surroundings/neighbours of the spin system. Longitudinal point out that it is the relaxation of magnetization in the $z$-direction which is the direction of external field. As shown in paper II paper fig.2.a. term $T_1$ is temperature dependent.

Spectral density and time correlation function (TCF)

Let’s start with the time correlation function TCF ($C(\tau)$) which is given according to equation (2.7) [1,2]. The TCF is also an autocorrelation function as it’s the stochastic process with itself at different times:

$$C(\tau) = \langle \gamma(t) \gamma^*(t+\tau) \rangle$$  \hspace{1cm} (2.7)

Here $\gamma(t)$ is a stochastic complex function in time, $\tau$ expresses the time difference, and symbol $<...>$ stands for ‘average’. Below fig.2.1.4. illustrates the time-dependent stochastic function and the corresponding TCF with two type fluctuations.

![Time correlation function (TCF) illustration](image)

Fig.2.1.4. The figure illustrate the fast fluctuation with a short correlation time (a) and slow motion with longer correlation time (b).
After a relatively 'long' time the correlation function will approach zero as $\gamma(t)$ and $\gamma(t+\tau)$ do not correlate anymore, then assume $<\gamma(t)>=0$, the quantity $C(\tau)$ becomes:

$$C(\tau) = C(0) \exp\left(-|\tau|/\tau_c\right)$$  \hspace{1cm} (2.8)

Here $\tau_c$ is the correlation time. The term $<\gamma(t)>=0$ doesn’t have to be zero for a stochastic function. As in relaxation theory it is often assumed that the average of fluctuation is treated as the isotropic average interaction so generally the remaining fluctuation interaction has a 'zero average'; and therefore the correlation function always starts at $C(0)$ and decay towards to zero (fig. 2.1.4.) by using equation (2.8).

$$J(\omega) = \text{Re}\left[2\int_0^\infty C(\tau) \exp\left(-i\omega\tau\right) d\tau\right]$$

$$= C(0) \left[\frac{2\tau_c}{1 + \omega^2 \tau_c^2}\right]$$  \hspace{1cm} (2.9)

$$R_1 = \frac{1}{T_1} = J(\omega) + 4J(2\omega)$$  \hspace{1cm} (2.10)

From equations (2.7) to (2.10) [1.2] the longitudinal relaxation rate $R_1$ were calculated. It starts with a time correlation function (TCF) (2.8) to calculate the probability at time $(t+\tau)$ of a particular orientation. The term $C(0)$ is called the coupling strength which is related to $\gamma(t)$ in equation (2.7). Equation (2.9) shows the real part of Fourier transform of the time correlation function, which is the spectral density. This real part gives the relaxation and the imaginary part would lead to a signal shift; which will not be talked here. The function of Fourier transform is to translate a time-domain signal into its constituent frequency domain. The correlation time $\tau_c$ is affected by the tumbling rate of molecular, as the term $\omega\tau_c$ shown in equation (2.9), the range of frequencies ($\omega$) are influenced by $\tau_c$. The relaxation rate $R_1$ (equation (2.10)) is given in the terms of spectral densities, and the spectral densities are key concept in NMR relaxation theory since they may contains information of molecular dynamics, such as rotational and translational motions.
SBM theory and SLA/SL Theory

In this work, FFC experimental results are interpreted on the basis of two theories: SBM and SL. This section starts with a brief introduction, and the equations used in paper II & III will be explained subsequently.

SBM stands for Solomon-Bloembergen-Morgan theory. It was developed and named after its developers in the early 1960’s [2.13-15]. It is a theory of nuclear spin relaxation and influence of the electron spin relaxation. Paper II, for instance considered that the enhancement of relaxation rate between different protein samples which is due to a paramagnetic enhancement of the proton spin-lattice relaxation rate caused by electron spin relaxation.

As in paper II, the electron spin of the four Mn ions of the OEC cluster has been considered to have net electron spin of 1. The proton spin-lattice relaxation rate $R_1$ can be described by the SMB theory:

$$R_1^p = \frac{1}{T_1^p} = [\text{const}]_i \tau_c^x$$

$$[\text{const}]_i = \left( \frac{u_i h \gamma_p \gamma_x}{4 \pi r_{i,x}^3} \right)^2$$

Here $r_{i,x}$ is the distance between the nuclear spin and the x spin. In paper II x is electron spin and in paper III it indicates the quadrupole nuclei. The other physical constants have their common meaning: the permeability in vacuum $\mu_0 = 4\pi \times 10^{-7} \text{ NA}^{-2}$; $\gamma$ is the gyromagnetic ratio which I is for proton and x is for another; $h$ is the Planck constant divided by $2\pi$; and $r_{i,x}$ is the distance between proton and another nucleus.

The effective nuclear spin-x, e.g. spin-electron spin dipole correlation time $\tau_c^x$ is given by:

$$\tau_c^x = 0.1s_i^x + 0.6s_j^x + 0.3s_0^x$$

It contains three terms of spectral density functions which individually looks like:
When $\omega_x \gg \omega_I$, equation (2.13) is approximately to:

$$\tau_c^x = 0.7s_y^x + 0.3s_0^x$$  \hspace{1cm} (2.17)$$

These spectral density functions are indeed depending on two frequencies. From the previous equations, effective correlation times can be expressed in the terms of spin-lattice ($T_{1,x}$) and spin-spin relaxation time ($T_{2,x}$):

$$\frac{1}{\tau_{1,x}} = \frac{1}{T_{1,x}} + \frac{1}{\tau_R}$$  \hspace{1cm} (2.18)$$

$$\frac{1}{\tau_{2,x}} = \frac{1}{T_{2,x}} + \frac{1}{\tau_R}$$  \hspace{1cm} (2.19)$$

The term $\tau_R$ denotes the reorientation correlation times and it is expected to be much larger than the other two relaxation times in the same equation and therefore can be neglected in the theoretical line-fitting procedure.

According to the Redfield perturbation theory [2.16-19], the $x$ spin relaxation time is given by:

$$\frac{1}{T_{1,x}} = [\text{const}]_2 \tau_s \left( \frac{0.2}{1 + \omega_s^2 \tau_s^2} + \frac{0.8}{1 + 4 \omega_s^2 \tau_s^2} \right)$$  \hspace{1cm} (2.20)$$

$$\frac{1}{T_{2,x}} = [\text{const}]_2 \tau_s \left( \frac{0.5}{1 + \omega_s^2 \tau_s^2} + \frac{0.2}{1 + 4 \omega_s^2 \tau_s^2} \right)$$  \hspace{1cm} (2.21)$$
Relaxation time $T_{i,x}$ ($i=1,2$) depends on the characteristic correlation time $\tau_x$ and the interaction strength constant ($[\text{const}]_2$). In paper II, for electron spin relaxation, the $[\text{const}]_2$ is:

$$[\text{const}]_2 = \frac{[4S_T (S_T + 1) - 3] \Delta_{ZFS}^2}{5}$$  \hspace{1cm} (2.22)

Here $S_T$ is the electron spin quantum number ($S_T = 1$), and $\Delta_{ZFS}$ stands for the transient zero-field splitting (ZFS) interaction.

In the zeolite paper III, this constant for the quadrupole nuclei Al with spin number $5/2$ becomes:

$$[\text{const}]_2 = \frac{3\pi^2}{10} \chi_q \left(1 + \frac{\eta^2}{3}\right)$$  \hspace{1cm} (2.23)

Term $\eta$ is the asymmetry parameter of the field gradient, $\chi$ is the quadrupole coupling constant.

The validity of SBM theory can be verified by a Kubo term [paper II]. The Kubo term is defined as $\Delta_{ZFS} \tau_{ZFS}$, which is the transient ZFS interaction term $\Delta_{ZFS}$ multiplied by the correlation time. For a Kubo term $\Delta_{ZFS} \tau_{ZFS} \geq 1$ the SBM theory is not valid. In paper II provides a better fitting to NMRD profiles using the SL approach as compare to the SBM theory.

**Stochastic Liouville approach (SLA)** or SL theory, originally stated as “Swedish slow-motion theory” was proposed in 1983 when N. Benetis et al [2.20] presented a theory of nuclear spin relaxation in paramagnetic systems. The electron spin relaxation is then allowed to occur in the slow motion regime. Later J. Kowaleswski et al., [2.21] and P.O. Westlund [2.22] in 1985 continued work on this approach. The theory is general but was applied to nuclear spin-lattice relaxation caused by the dipole-dipole interaction between nuclear and other spins for example $S_e=1, 3/2, 5/2, 7/2$, etc.

Continued from the preceding SBM theory, when the modulation of the zero-field splitting (ZFS) (interaction) is due to slow structural fluctuation/change or by slow reorientation motion the SL theory is needed. The advantage is that SL is a general theory with no restriction in analysis. Further details of the theory are described by the related publications [2.4], [2.21], and [2.23].
Some limits:

![Image of proton T1-NMRD profile from extreme narrowing limit (ωτ_c << 1) to dispersion limit (ωτ_c >> 1); where the green line between black and red profiles are the point at ωτ_c = 1. The relaxation rate is normalized to 1. The figure is generated by a single Lorentzian function:]

\[ I(\omega) = \frac{r(0)}{1 + (\omega \tau_c)^2} \] (2.24)

Here r(0) is setting to 1.

In the theoretical proton NMRD profile (fig.2.1.5.), on the left hand side is called ‘extreme narrowing limit’, where ωτ_c is much smaller than 1. When the profile gets to the extreme narrowing limit the relaxation rate reaches a value independent on the Larmor frequency. If applying this limit together with the Redfield regime, the relaxation times T_1 and T_2 in phenomenological Bloch equations become equal.

The right hand side is the ‘dispersion limit’ where ωτ_c is much larger than 1. For a single Lorentzian (2.1), we observe dispersion and there will be one frequency that is equal to the invert of the correlation time τ_c when ω = r(0)/2.
2.2 Molecular Dynamics (MD) simulation

Molecular motion

In this thesis, the main work is focused on analysis of the molecular motion in liquids. In liquids, molecules have more freedom to move compared to the solid phase. However, even in solid molecules are not totally immobilized since atoms are always vibrating/oscillating. These vibrations are very fast, typically on sub-pico second (ps) scale. Bigger molecules could show relatively slower vibration [1.11].

In liquid molecules can rotate randomly. These random rotations change the anisotropic spin interactions such as the spin dipole-dipole couplings and can therefore influence NMR measurable quantities. The time range may vary due to the liquid viscosity and the temperature. It has been reported that small molecules in liquid reorient in ps range, and bigger molecules can be much slower (ns [2.24], µs [2.25], ms [2.26], etc.). The simple approximate estimation of characteristic time range can be calculated by using the molecular mass with the equation \( t = \frac{M}{2} \) [1.11]; where \( t \) stands for the rotational correlation time of a globular protein in room temperature water, and \( M \) stands for the molecular mass in kDa. For example, a protein of 100 kDa maybe have rotational timescale in water of 50 ns. Macromolecules such as proteins normally have slower motion compared to small molecules in liquid.

Another type of motion is translation. There are two types, self-diffusion and flow [1.11]. Translational diffusion is a random walk, or irregular type of molecular movement; and flow has a concerted direction. Molecular translation has two effects on NMR properties both on the microscopic and macroscopic scale. On the microscopic scale, molecular diffusion averages the short range intermolecular spin interaction. On the macroscopic scale, flow/diffusion can carry molecules from one area to another and this will affect the spin behaviors in the inhomogeneous external magnetic field. NMR can detect and distinguish between these two translational motions [2.27-28].
MD simulation

Molecular dynamics simulations are computer based calculations which provide information about the molecular motions of these systems. MD simulation has been used to study the properties of assemblies of molecules to get atomic information about microscopic interactions (between them). The method generates configurations from dynamic evolution of atoms and helps to understand the physical basis of the biomolecules; and their important function in organisms [2.29-34]. Since McCammon et al., [2.31] presented the very first MD simulations of a folded globular protein (bovine pancreatic trypsin inhibitor, BPTI) and with forty years the methods have continually developed. Now MD simulations are successfully carried out on various systems in thousands of publications. [2.35-37]

MD is used to evaluate motions of atoms by numerically solving the classical Newton’s equations of motion with conserved Hamiltonian of the system. Pressure and temperature are two important factors need to be controlled in realistic experiments and it is the same for simulated systems. There are several ways to provide temperature control, for example the Nosé-Hoover thermostats [2.38-39] and V-scale thermostats [2.40]. The Berendsen barostat [2.41], Andersen barostat [2.42], and the Parrinello-Rahman barostat [2.43] are developed for constant pressure control. Details of those methods can be found with its references and will not be discussed in this thesis.

As a fundamental tool MD simulations could provide details about motions as a function of time for a particular molecule and provide an approach to describe its behaviour. Firstly a model system (for example protein PrxV in paper I) will be selected in the study and get started by using an X-ray crystallographic structure (normally a PDB file) of good resolution. A good
resolution helps to gain the clean structural information of the selected protein. Then hydrogen atoms and side chain atoms are added as appropriate if they are missing. The time evolution of bond angles, lengths, angular torque, charges, and etc., are described by force fields, which contains equations and constants to reproduce the molecular properties and geometry. Force fields contain the potential energy functions and corresponding parameters which are integrated with Newton’s law of motion to obtain trajectories (MD trajectories) [2.44].

Classic MD simulation cannot handle chemical reactions since it needs quantum mechanics to describe charge transfer etc.

![Fig.2.2.2. Snapshots (time gap = 2.5 ns, total time length = 20 ns) shows the dynamics of the protein structure of PrxV including colourful selected water molecules from MD simulation; blue colour indicate water with residence time between 2 ns to 5 ns, yellow colour indicate residence time between 5 ns to 10 ns, and red colour indicate residence time larger than 10 ns [2.45]. The figure is reproduced from complementary material of paper I, and courtesy to co-author Dr. Nam.](image)

As shown in fig.2.2.2., MD provides information which cannot be obtained from other methods (such as X-ray crystallography). Those selected water (Wn: crystal waters or Bn: bulk waters) molecules are β type water with longer residence time from 2 ns to 20 ns which W1 is the crystal water and B1 to B4 are added bulk waters. More discussion on this topic will be given in summary paper II.

The simulation method also provides more flexibility as it is free to change the setting parameters to investigate multiple hypotheses; as in nature you
may not be able to modify the experimental method very much due to technical reasons or costs.

Computer simulations are based on mathematics and modern computational techniques, it may not exactly descript the behaviour of real world but offers a supplementary way to predict the results by test different simulation parameters. MD simulation is very illustrative and offers a new way to look into details of ‘experiments’.

The power of computer science grow quickly, programs for MD simulation (such as NAMD, CHARMM, AMBER, GROMOS, etc.) are updated fast and regularly. Now a days the simulation time has extended to range from ns to ms. For example B.Halle et al., [2.46] has recently published a research with the aim of explaining how amide hydrogens exchange in native proteins. The study is based on an ultra-long MD simulations that correspond to 0.262 ms. The elongation of MD simulation times make it possible to investigate biological phenomena as they happen.

**Analysis of MD trajectory**

In MD simulations, one gains motional trajectories. Trajectories are sets of time dependent (x, y, z) coordinates of molecules. As an example, consider the atomic model with 160 identities (as in paper IV):

![Fig.2.2.3. The atomic model with 160 proton identities. Black and green lines indicate the paths to form vectors.](image)

The black squares in the figure above indicate a selected atom (can start with any one of those 160 protons), and the red dots (the rest 159 protons). For each time frame formed 159 dipole-dipole vectors in between, and those
vectors with the lab frame (0, 0, 1) will form a particular angle $\theta$, and a distance $r_i$. The stochastic quantity $h^{DD}$ is defined as:

$$h^{DD}(i) = \sum_{r_i} \frac{[\frac{3}{2} \cos^2 \theta(t) - \frac{1}{2}]}{r_i^3(t)}$$

(2.25)

; a factor of 2 is reduced in the calculations as the relationship has counted twice. For example atom (1) $\rightarrow$ atom (2) is the same as atom (2) $\rightarrow$ atom (1).

A time correlation function is calculated as follow:

$$c(j) = \frac{1}{N + 1 - j} \sum_{i=1}^{N+j} h^{DD}(i)h^{DD}(i+j)$$

(2.26)

Here index $i$ and $i+j$ indicates the time steps, $N$ is the number of total stochastic quantities.

Fig.2.2.4. shows a typical correlation function. The highest value is normalized to 1. The decaying tail is used to calculate correlation times. In this figure the inter-molecule correlation time is 45 ns and for intra-molecule it is about 30 ns. Plot in blue colour is the cross term between intra- and inter--; as the value is almost zero therefore we consider that term is very weak and can be ignored.

Fig.2.2.4. Figure shows a proton-proton dipole-dipole correlation function from paper IV. Intra- in black, inter- is in red and the blue is the correlation term. The upper figure is normalized to the range between [0, 1]; and the lower figure is the logarithm plot.
MD simulations and NMRD

As previously mentioned, MD simulations rest on classical physics and do not include quantum mechanical properties. The NMRD is based on a quantum property: the concept of spin. Could one use these physical theories to interpret the same problem? The answer is yes since they are combined together to study the dynamics of a system. In one hand, MD simulations can be used to calculate motional correlation functions and provide details about each selected molecule; on the other hand relaxation rates offer an ensemble averaged result of the dynamics of the whole macroscopic system.
2.3 Fast-field cycling (FFC): Experimental method

We have used a 1 T Stelar FFC 2000 instrument from Italy to study the $R_1$ relaxation rates of different samples. The FFC instrument has been successfully used as a tool in widely varying research topics [2,47-50]; such as biomedical (MRI contrast agents, protein studies, etc.), materials science (polymer material, etc.) food science (quality control of foods, etc.) Mainly FFC experiment is used for proton but it is also suitable for heteronuclei such as deuterium, fluorine etc. As a non-destructive technique FFC NMR relaxometry does not destroy the sample itself and therefore can provide flexibility for other experimental purposes. Fig.2.3.1. shows an example of NMRD profiles obtained by a FFC instrument at Umeå University which are reprinted from paper I.

![Graph showing NMRD profiles](image)

Fig.2.3.1. The proton $R_1$ NMRD profiles of intact PSIIcc from *T.Vulcanus* obtained at 10 °C at four different PSII reaction centre concentrations (29 µM in black squares; 60 µM in red circles; 123 µM in green triangles and 188 µM in blue inverted triangles). Figure reproduced from paper II.

On the left side of the coordinate gives the relaxation rate $R_1$ in the unit of s$^{-1}$. The relaxation rate is measured as a function of the magnetic field (the Larmor frequency) which normally given in MHz. In our results it often present in the range from 0.01 MHz to 40 MHz for proton experiments.
**How to obtain the relaxation rate $R_1$ with the FFC instrument?**

A typical experiment is set up with 30 selected frequencies for measuring the relaxation rate, $R_1$. The pre-polarized method [2.51] is fairly important in this technic because the longitudinal nuclear magnetization is increased by using this method and thus the signal intensity improves dramatically.

![Diagram](image)

Fig.2.3.2. The basic scheme of a pre-polarized sequence. The green area indicate the magnet switch-off/on time. The free induction decay (FID) is an oscillating signal generated by the motion of the magnetization vector in the detector of the NMR spectrometer; and the decay of FID is the origin of NMR. And a 90 degree RF pulse is used to turning the magnetization. (This figure can also refers to Bloch equation 5: polarization field $M_0 \rightarrow$ variable times $\rightarrow$ obtain $M(t)$.)

According to fig.2.3.2., relaxation delay is varied with typically 16 different times per relaxation field. And normally the FID picked up 1000 points for a specific relaxation field and specific relaxation delay (variable time in the figure). Thus the complete dispersion is recorded by acquiring at least $16 \times 30 = 480$ FIDs. Moreover each FID can be repeated a specific number of scans to obtain proper good signal to noise.

The method follows a polarization $\rightarrow$ relaxation $\rightarrow$ acquisition procedure. In order to improve signal intensity the sample is pre-polarized (normally is done at 25 MHz); then the field is lowered and the sample is allowed to relax during a certain time; finally the field switched to the detection field (16.3 MHz) for signal acquisition [2.52]. As compare with the natural signal strength at the lowest field of 10 kHz, the FFC pre-polarized signal strength becomes millions times ‘stronger’ than the natural signal strength. So without the method it is not possible to measure $R_1$ at low fields. For higher fields (>12 MHz), we use a non-polarized method. The method has no spectral resolution and it simply measure the intensity of the solvent signal as a function of relaxation field and relaxation delay.
2.4 NMRD profile fitting procedure

From MD simulation

In paper I MD trajectories were analysed by FORTRAN programs for extracting information about NMRD models (details see appendix C). We begin with a NMRD profile from paper I:

![Figure 2.4.1](image)

Fig. 2.4.1. The stretched NMRD profile with $\tau_R = 50$ ns (in solid line) and analysed by using a single Lorentzian function (in dotted lines a, b, c). The figure is represented from paper I.

In this particular fitting procedure one starts with a single Lorentzian function by equation (2.24) with $r(0) = 1.4$, and the correlation time $\tau_c$ are 20 ns (a), 10 ns (b), and 5 ns (c).

The equation (2.24) offers a very simple relationship between the observed results (both frequency and highest relaxation rate $r(0)$) and only one uncertain factor, $\tau_c$.

The fitting is carried out from low to high fields, and three results (a, b, and c) clearly reveals that a single Lorentzian function cannot fully explain the stretched NMRD profile. Each of them could only fit to a limited range of frequencies of the profile.

If we have more than one unknown parameter, for example, take $x = a * b * c * d$ and only $x$, a, and b are given values; in case $cd = x / (ab)$, and the values of c and d can be varied for a large ranges as far as the multiplication equals to
To avoid this problem, there are some validities in the models which we used for analysis experimental results. For example as in Paper II, if the Kubo term becomes much smaller than 1 then the time dependent perturbation Redfield theory becomes applicable. For the validity in using the Redfield theory the Kubo term should be kept \[2.4-6\]. Thereby the fitting range has been limited. In order to control the quality of fitting procedure a chi-sequence test was performed. Table of parameters presented in previous publications are also good resources as references.

**Fig.2.4.2.** The stretched NMRD profile (same as fig 2.4.1., black solid line) with \( \tau_R = 50 \) ns (squares) is displayed with fitting NMRD profiles (red solid line) calculated using the NMRD model of equation (2.2), using three effective correlation times \( \tau_c \) (0.7 ns, 4.0 ns, and 12 ns).

As compared with the fitting procedure (fig.2.4.1.) of a single Lorentzian function, the information of a stretched NMRD profile is well extracted and a multiple parameters model (e.g. three different correlation times; which in a single Lorentzian function only one correlation time is needed) can be used to reproduce the experimental NMRD profiles.
Fig. 2.4.3. Experimental deuterium T1-NMRD profile (black square) of a Zeolite sample at 50 °C with theoretical fitting (in red line, a & b). The fitting parameters are $\alpha = 18.9$ (in green line), $\tau_{c,1} = 3.1 \mu$s, $\tau_{c,2} = 0.25 \mu$s.

The fig. 2.4.3. presents a deuterium T1-NMRD profile obtained for a zeolite sample (paper III). As shown in dash lines, it is made of two single profiles each fitting for a certain field region. A constant relaxation rate $\alpha$ is given as a fitting parameter as it does not vary with the frequency.

The first step is to start with the high field region (looking for the correct profile a), as the scaling factor is unknown one should try to follow the experimental results at high field as much as possible. Then with the help of the first theoretical profile we could add another one in the low fields. Now the scaling factor consist of the sum of two profiles and should be fitting to a constant relaxation at the low field limit.
Some details of the fitting procedure

This section is aiming at explaining some details of the fitting procedure.

Fig.2.4.4. Theoretical proton T₁-NMRD profile from a simplified model by using equation 2.3 with a correlation time of τₑ = 10 ns (black), 50 ns (red), and 100 ns (blue). Fitting are normalized (b) to a constant R₁ rate = 1.0.

Equation (2.27) is a simplified version of equation 1 in paper III for describing the intra-molecular spin-lattice relaxation rates R₁ of water proton; where ω₁ is the proton Larmor frequency and τₑ is the water reorientation correlation time. Dipole-dipole coupling constant and scaling factor and etc. are skipped; it only depends on the frequency and the correlation time. With the fig.2.4.4.b it is clearly that the dispersion shifts toward to lower frequency region with using higher τₑ value.

\[ R₁ \propto τₑ \left( \frac{0.2}{1 + \frac{ω₁^2}{τₑ^2}} + \frac{0.8}{1 + 4ω₁^2τₑ^2} \right) \]

(2.27)
3. Summary and Discussion

Paper I

In this paper the water proton R1-NMRD profiles of a hydrated protein PrxV (fig.3.1.1.) are analyzed with a generally accepted relaxation model (details see Appendix C). The model has been used for describing various NMRD profiles of different proteins. In contrast to other publications, the NMRD profiles analyzed here are extracted and reproduced from MD simulation trajectories; which simply named as 'exact' NMRD profiles.

Fig.3.1.1. The structure of PrxV at a resolution of 1.5 Å. PDB data from reference [3.1] were reproduced with VMD software.

We select PrxV as a model system for three reasons: it is small sized; it has a high resolution crystal structure below 1.5Å which allowing us to identify water molecules nearby; and the protein is ubiquitous and dominant antioxidant enzymes which is very interesting to work with [3.2].

The total MD simulation time is 20 ns, and the system was solvated with a rhombic dodecahedron (RHDO) box that contains 2993 (Nw) (the total number of) water molecules. The coordinates of the water molecules were saved every 1 ps during simulation.

From traditional experimental methods, such as results obtained from different spectroscopic techniques it is hard to distinguish between different types of water-biomolecule interaction. With the MD method it is possible to identify the type of waters according to their residence time. Two types of waters have been determined, and therefore water molecules are separated into two groups: α and β water.
The β group contains water molecular with longer residence time in the range of 0.5 ns to 20 ns; whereas those with residence time less than 0.5 ns are diagnosed as α group water. These α water molecules exchange rapidly with bulk water and in case they also referred to as being ‘surface’ waters. The β group, residing within the protein structure with few or no neighbours can be named as ‘buried’ waters. The average residence time of the surface waters is 26 ps. According to the NMRD model from reference [1.17, 3.3-4] α water molecules give a field-independent relaxation contribution, we only focus to discuss β water molecules as they give rise of NMRD relaxation dispersion profiles.

Plenty of α-waters and a small amount of β-waters were determined. With the help of MD movie (available from publication webpage), it revealing that not all crystallographic waters are long-lived β-waters; and actually lots of crystallographic waters leave their positions and exchange with the bulk water.

Fig.3.1.2. The distribution map of residence time obtained from MD simulation trajectories displays the number of β water molecules with its residence times in the interval 0.5 < τω < 20 ns (a); and the distribution of residence time from ‘exact’ NMRD profiles obtained from the MD simulation (b). The figure is reproduced from paper I and corresponding to table 1.

The fig.3.1.2. shows two distribution maps (a and b) of residence time; where the population number of β (Nβ) water vs. its residence time (τω). We use 0.5 ns as the time interval to group them. By using NMRD profiles (b) are only obtained a much simplified picture (b has less columns compare to a) compared to the one obtained directly from MD simulations (a). The information obtained from the NMRD analysis is an average over those waters with similar τω. By using MD one can track every interesting water molecular during the whole simulation time and get exactly the individual
residence times. Thus the distribution map contains more information from MD (a) which cannot be reproduced by NMRD (b).

From the distribution map (a), we input every calculated residence time from 0.5 to 20 ns to produce ‘exact’ NMRD profiles (equation 5, paper I). In this way we construct ‘experimental’ results.

![Image showing NMRD profiles and order parameters](image)

**Fig.3.1.3.** The “exact” NMRD profiles obtained from the MD simulations with different reorientational correlation time $\tau_R$ of 1, 10, and 50 ns; with a theoretical line fitting using different parameters (a); and the order parameter $S_0$ for $\beta$ waters against residence time $\tau_0$. The figure reproduced from paper I, and detailed equations and parameters are given by equation (2), equation (5) and table 1.

The calculated order parameters are approximately close to 1 (fig.3.1.2.b) for most $\beta$ waters hence the relaxation contribution to the dispersion is ignored as the fast local reorientation is not counted. By checking the order parameters, $\beta$-waters showing larger order parameters dominate the main relaxation contribution. The causes of short order parameter are due to local protein fluctuation and not the local water motion. The residence times extracted from (b) are shorter than those from (a) which suggests that the information of (b) gives an averaged overall picture of waters with similar dynamics.

From a protein side review, it also found that some protein binding sites not always binding with the same residence time which suggest that the local structural fluctuations could effectively change the local water dynamics. Additionally, this NMRD-MD method is used to evaluate the information content of the NMRD profile but not for the purpose of giving any quantitative conclusions on the water dynamics of the protein.
Photosystem II (PSII) is the place where photosynthetic water oxidation occurs with chemical energy and oxygen as products. The light-driven water-splitting procedure is carried out by the oxygen-evolving complex (OEC) (fig.3.2.1.). More than 1300 water molecules were identified in each PSII monomer [3.5]. Study the binding of substrate water to the Mn₄O₅Ca cluster provides crucial aspects on the mechanism of photosynthetic water oxidation.

Fig.3.2.1. Figure shows an OEC complex (black circle and square; with Mn₄O₅Ca cluster structure: Mn in purple, O in red, and Ca in green) in the overall structure of PSII dimer from *T.Vulcanus* at a resolution of 1.9Å. Figure reproduced by writer from .pdb file of reference [3.5].

For a better understanding of the hydration of the OEC complex in the dark stable S₁ state of PSII proton T-NMRD profiles were used for the characterization. Experiments were analyzed both with intact and Mn-depleted protein samples at four PSII reaction center concentrations from 29 µM to 188 µM. Experiments were performed at three temperatures. And frequency ranges from 0.01 MHZ to 40 MHz by a fast field-cycling instrument.
It is found that the relaxation rate $R_1$ at the lowest field is approximately proportional to the concentration changes. However in low frequency region appears small digressions at the higher concentrations and this may be due to the increase of inter-complex interactions of the complex, which would delay the dynamics and therefore increase the relaxation rate. The relaxation rate also increases at decreased temperatures which indicates a fast exchange between bulk protons and the PSII associated protons. The sample of the 60 µM is chosen for discussion.

The green profile in fig.3.2.2. is the one we are interested with. Firstly the idea is to test whether there is a paramagnetic contribution of the cluster to the dispersion profiles. There are two types of water molecules according to the relaxation theory, either surface water (α water) or water buried inside the protein (β water). It is possible to fit the profiles assuming paramagnetic enhancement of the $^1$H-relaxation however this requires an exchange of large number β water molecules. What may happen after the removal of Mn? For native sample 360 β water molecules were identified; and this number has decreased to 230 for Mn-depleted sample with the same concentration at 60 µM. In the case that the Mn elimination procedure did not alter/change the protein structure, the hydration with β waters should be larger after Mn-depleted compare with intact PSII as more water molecules can penetrate into the cluster cavity. This hypothesis is in contradiction to our results (230 < 360) and therefore can be excluded. On the other hand loss of extrinsic
proteins is also expected to lead to the observation of less β waters for Mn-depleted sample. Without any direct experimental proof, it is considered that the difference is mainly caused by the paramagnetic enhancement of the cluster.

Then (intact minus Mn-depleted, green in fig. 3.2.2.) NMRD profiles were analyzed by SBM and SL approaches. In the SBM theory the Kubo term needs to be much smaller than 1. However, the simulation result (table 2 in paper II, see the Kubo term) shows that this is not the case. Thus need to use the more generalized slow motion theory. Both SMB theory and SL theory cannot describe the small increase of R1 at about 20 MHz with only one g-value. In contrast, a ‘good’ fit is acceptable by using two g-values with SL theory (fig. 3.2.3.b).

Fig. 3.2.3. The line fitting of the experimental water proton R1-NMRD difference profiles of the OEC complex using the SL theory with one g-value (a) (g = 4.9) and two g-values (b) (g = 4.9 & g = 2.0). The square is 123 µM sample and the triangle is 188 µM sample.

In conclusions of this work it is strongly suggested that electron spin relaxation makes an important contribution to the relaxation time. Based on the SBM theory and the SL theory the difference dispersion profile is due to a paramagnetic enhancement of 1-2 water molecules nearby about 10 Å nearby the cluster; and these water molecules could exchange with the bulk in sub µs time scale. The reorientation modulated by the ZFS interaction of the cluster has characteristic time about 0.6 to 0.9 ns. It is found that the SL theory can be used to interpret the difference profiles better than the SBM theory as it is more general and has no perturbation criterion.
In this paper, both the water proton (\(^1\)H) and deuterium (\(^2\)H) NMRD profiles of fully hydrated ZSM-5 zeolites (fig. 3.3.1) were analyzed in order to explain the water dynamics in the confined space of zeolite.

Fig. 3.3.1. Figure shows a TEM scan of ZSM-5 sample used in our NMRD analysis. Apparently it is seen that the sample get aggregated together. (Special courtesy to Dr. Cheng Choo Lee for TEM experiments.)

In experiments of sample preparation, we have used calcination method to eliminate nitrogen from the original sample to create H-ZSM zeolite. Surprisingly that even with a very long calcination over 12 hours still cannot reduce the content of nitrogen in ZSM-5. This observation maybe explained by the pores are rather structurally curved and flows may not reach the centre areas.

There are two forms of ZSM-5 have been tested, the ammonium form (NH\(_4\)-ZSM-5) and the proton form (H-ZSM-5). Experimental results of proton and deuterium R1-NMRD profiles are shown in fig. 3.3.2.
From proton experiments, in the low field region and at higher temperatures larger relaxation rates (a) were found in the H-ZSM-5, but almost no temperature dependence for NH$_4$-ZSM-5 (b). The proton relaxation rate of H-ZSM-5 is higher than NH$_4$-ZSM-5. And there is a strong increase at high frequency (start from ~20MHz) for proton experiments however this present absence in deuterium experiments (c, d).

It has been reported that this zeolite has micropore size of ca. 5 - 6 Å [3.6] according to their formation templating mechanism. Hsu et al., [2.48] reported a three-site model to interpret the dynamics of water in the pores of zeolite H-ZSM-5 and formed three different pore sizes ranging from 0.5 nm to 50 nm. The BET (appendix B.1 & B.2) experiments show beyond the micropore size there are also larger pores. A TEM scan (appendix B.3) and found that in addition there are also big pinholes. The cause of those larger pores may come from the impurity of the sample as it is bought commercially.

The experimental profiles were analyzed by applying a simple relaxation model. The correlation time is assumed in the ns range for water spin-lattice relaxation profiles; and two or three theoretical profiles were needed to fit the experimental dispersion. The main idea behind the dispersion concerns confined water in those different narrow zeolite pores. And different counter
ions (H⁺ or NH₄⁺) could effectively change the water translational diffusion in the pores and also change the hydration. The stretch NMRD profile also indicate that there are different sized pores.

The fact of multiple pores observation in experiments is consistent with information achieved from NMRD profiles, because the theoretical line-fitting indicates two or more characteristic correlation times which suggest a certain distribution of pore sizes. However it is not possible to relatively point out its correspondence with specific pore size. And if it is too big (such as 40 to 50 Å), too much bulk water molecules can go into it and the relaxation enhanced may be decreased. Fig.3.3.3. shows theoretical line fitting to experimental results. Two profiles have been combined to produce the line fitting, and indicate the correlation times are in µs range for NH₄-ZSM-5 samples. One obtained correlation time is about 1.3 - 3.1 µs which is about 5 to 12 times larger than the other one (0.25 µs). However the correlation time obtained from theoretical analysis to NMRD profiles NH₄-ZSM-5 samples has ns and µs time range.

Fig.3.3.3. Experimental deuterium R1-NMRD profiles of H-ZSM-5 and with theoretical line fittings (a) and (b) a comparison between sample NH₄-ZSM-5 (red) and H-ZSM-5 (blue). Red triangle = 30 °C, green square = 50 °C, and blue star = 70 °C. Figure is reproduced from paper III, and fitting parameters can be found in table 5, Paper III.

The analysis shows that the proton and deuterium NMRD profiles may be used as a good tool to characterize different zeolites.
**Paper IV**

Ionic liquids (IL), it’s a class of organic salts which has their pure state in liquid at/near room temperature [3.7]. It belongs to a class of materials which have low viscosity, better reactivity, a better selectivity compare to organic solvents. Moreover it is an environmental recyclable material.

![Molecular Structure](image)

Fig. 3.4.1. The molecular and chemical structure of IL BMIM\[PF_6\]. CNOP indicate the four united group in the coarse-grained model of MD simulation. Figure is reproduced from paper IV.

This paper studied the molecular reorientation of molecules by using proton and fluorine spin-lattice NMRD measurements for IL 1-butyl-3-methylimidazolium-hexa fluorophosphates (BMIM\[PF_6\]) (fig.3.4.1.) at different temperatures. Measurements were carried out at frequencies ranges from of 10 kHz to 40 MHz and at different temperatures and different nuclei (fig 3.4.2). And both atomic and coarse-grained (CG) trajectories by MD simulations at 323 K were used in the interpretation of the NMRD profiles.

![NMRD Profiles](image)

Fig.3.4.2. R1-NMRD profiles for proton (black plots) at three different temperatures (square = 50 °C, dot = 5 °C, and triangle = -5 °C) and for \(^{19}\)F in red plot (star = -5 °C).
From fig.3.4.2., it is seen that the relaxation rate $R_1$ of $^1$H experiments is higher than for $^{19}$F experiments. However the line shapes are very similar. We have analyzed the presented results with a simple relaxation model based on the intra- and inter- molecular dipole-dipole (DD) relaxation mechanism (fig.3.4.3.). The results from theoretical line fitting indicate that correlation times in the range of ns (10 ns, 66 ns, and 620 ns (see table 1 in paper IV for all parameters). In both proton and fluorine NMRD experiments the effective correlation time are similar.

The dipole-dipole correlation functions were calculated based on atomic and a CG MD simulations. It is found that the reorientational dynamics of BMIM$[PF_6]$ and BMIM$^+$ ion are in the ns time regime (it is 45 ns for proton intra- and 30 ns for inter- molecule for atomic simulation) whereas the reorientation of $[PF_6]$ is in the time range of ps. Based on the MD simulation we performed so far, the picture carried out from MD is consistent with the NMRD results. However the density of charges in IL may cause problem due to the slow long range correlations; for which the time of the MD trajectory is not long enough to pick up the slowest motions (e.g. the 620 ns) affecting the NMRD profiles.

The information extracted from MD simulations is compared with that obtained from NMRD profiles. It is found that they give very similar time scales. The ion pairs can stay 4 - 6 ns on average according to simulation and in FFC experiments the reorientational correlation times are all in the same time regime. Therefore NMRD profiles are indeed informative and offer significant way to study the dynamics of ionic liquids.
4. Conclusion

This thesis summarizes the analysis of NMRD profiles in different complex systems, and the following conclusions are drawn:

In this thesis we give a general review on analysis of NMRD profiles; together with the analysis of MD simulation trajectories. To the best of our knowledge, it suggest a pioneer analysis which combines MD simulations results and NMRD together to reveal more information than an individual approach. By NMRD profiles one could extract an averaged information over water molecules with similar dynamical behaviour; and full details may be extract only from the MD simulations. However, the results of MD simulation rather dependent on the simulation length.

It state that the NMRD profiles are very useful hereby to acquire a quick review of the system, and by using MD methods more detail information will be carried out.

The SBM theory and the SL theory were both used to interpret the paramagnetic enhanced relaxation of water. The slow motion theory has an advantage which it could cover the range where the SBM theory is out of its validity; such as in paper II and paper III.

It is very interesting to study NMRD profiles with different nucleus, such as $^1$H, $^2$H, and $^{19}$F. They can complementary provide information of dynamics between the solvent and target (such as the protein).
5. Future work

In the previous discussions we have mentioned the advantage of MD simulations, which could be used as a complementary resource for the analysis of the NMRD profiles.

In paper I it may also very interesting to look at the experimental NMRD profiles of protein PrxV.

In paper II, it is not clear whether the structure has been changed during the elimination of Mn and we could only provide prediction from theoretical models. MD simulations may clearly answer the question as it offers direct illustrative information. D.V.Singh et al has presented a molecular modelling and computation simulation of PSII reaction centre in *Phalaris* minor [4.1]. The similar research may also be extended to our species *Thermosynechococcus.Vulcans*.

Since the results of MD simulation are dependent on the length of the simulation, further MD simulation in a longer time length can be planned for both OEC (II) and IL (IV) projects. Then it is possible to test whether the analysis of model is consistent. D.Tranca and F.J.Keil have reported an *ab initio* molecular dynamics study of chemical reactions to describe the dynamics of hexane cracking in zeolite ZSM-5 [4.2]. It is reasonable to predict that the full simulation will be carried out sooner or later with the improvement of computer science.

In OEC paper (II) we assume that the reorientational motion of the metal cluster is independent of the reorientational motion of the whole protein complex and this may be further verified by quantum chemical calculations. We did the analysis in S1 state, and it may extend to other states (e.g. [4.3]); also extend to other species such as spinach and etc.

In zeolite paper (III) our analysis have shown that only the SLE theory can solve the relaxation problem and this would be an interesting topic to continue work with.

It is popular nowadays to deal with cross subject problems, such as chemistry/physics, biophysics, and etc. NMRD and MD are both intensive tools in measurement and to test hypothesis. The future I believe that NMRD-MD analysis will be extended to other similar topics such as the study of protein hydration.
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First of all I would like to thanks Prof. Gerhard Gröbner who introduce me to my supervisor since I was a master student; otherwise I may have no chance to be a student of him.

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To my spiritual father von Clausewitz:

We met each other when I was a little boy; in the military topics taught by the private tutor Dr. Tian. Years by years, I could understood what you've written in those great books. To be born in a clan of 'wolves', it is your wisely guidance that keeps me always go straight forward; and to survive all those Spartan-style cruel trainings added to me in my younger days.

To dear ‘papa’ Bach:

Great songs forever!

And to my biological parents: No matter you like or not, and no matter that as parents you never hugged your only son; still, thanks for give birth me. You provided me a very unique and impressive childhood.

何でも、僕を産んでくれてありがとう。

Finally, I would present my deepest appreciation to my supervisor Prof. Per-Olof Westlund, who taught me since I was a master student. He introduced and supervised me to a very interesting and promising topic. In the past four years, we did six projects together. Beside those four I have talked in the thesis, we did another two projects with ganglioside micelles and a theoretical work in the relaxation of the singlet state of water. Due to the experimental results and the lacking of time, they are not ready to present yet. But I do have lots of fun things to play with; thanks! And also thanks him to translate the abstract into Swedish!
Private thanks to dear ‘minstrel’, the artist who draw this paint for me: Thanks for support me in the past 23 years. 感谢你在过去的二十三年里无条件地尊重并支持我的信仰及理想;
“
安得青天化作一赋纸，有长笑白首苍山，我倾国之寄千里。”

Now I have written a lot for the others; finally I’ll write a little bit about myself to summarize all my school days:

Full Name: Zhen-Yang (振洋) Henry Huang; use school name as Yang Huang.

Born: Nov.14th; 1986, to Mr. Henry Huang and wife.

Self-identification: scholar, musician, origamist, programmer, and collector of fine ancient arts.

Short biography:

I was born as the first and the only child to a direct male-line descendant of some historical figures; and I was expected to become a classical pianist by the wishes of the very many within the house by birth; or to become a military leader by the father’s private willing. As a ‘professional’ bookworm and a heavy reader since 2 years old, I left from both pearly wishes due to changed self-interesting on nature science.
I started my piano training before my third birthday, and still play it today. Together with General von Clausewitz, musician J.S.Bach is also another most remarkable talent I appreciated so much. The first giant taught me the way to survive in this tough world; and the later giant proofed me that joyful can be found anywhere if you carefully listening to the marvelous nature; indeed.

My first scientific work has been carried out when I was about 10 years old, as I proved the Fermat’s little theorem individually. I was so proud of it and simply name it after my name with the over self-confidence of a so-called ‘whiz kid’. (However one of my governesses later pointed out that this has been done long time ago.) Since then, I keep a lifelong curiosity with the number theory. My favorite numbers are prime numbers, plus 0, 1, and 137.

I’ve got my first personal computer in 1992 and apparently became a programmer since then. By 11 I entered into a boarding school specially made for those selected whiz kids. I’m bored with a typical whiz kid’s well planned elite-style life procedure, after that I has been sent to Denmark at the age of seventeen. When I finished two bachelors in Chemistry & Molecular biology, plus another one year in computer science and another one semester in human science (in the topic of Mongolia history) there, I moved to Sweden to continue master level study (completed in 2011) and so to a PhD. As grown in an international environment, I generally speak English, Mandarin Chinese, some Japanese, some German and Danish (actually never have a chance to use it since I left Denmark!). For computer language, I familiar with LOGO, Visual Basic, PROLOG, Java, NScripter, Python, MATLAB, R, FORTRAN and also play with Android developer tools. I play tennis, swimming, distance running, Kendo, and ride horse for hunting. I wish to be a pilot since I was young, and currently training with the PPL courses.
7. References


[2.45] Paper I. Fig is originally created by co-author Dr. Nam. A supplementary movie is also available at the publication’s webpage:

http://pubs.rsc.org/en/Content/ArticleLanding/2013/CP/c3cp51147b#!divAbstract


(80 references in total)
Appendix A Technical Details

Math formulas are written by Visual Tex V8.42 and MathType V6.9a.

Figure is made by Microsoft Paint V5.1 & PhotoShop online.

Program uses Compaq Visual Fortran compiler. (FORTRAN 77/90)

Data analysis uses Origin V6.1 and V8.5.

Text is written with Microsoft Word.
Appendix B Extra experimental results

BET report for Paper III

Fig. B.1. An isotherm linear plot indicate the ZSM-5 sample contains different pores.

Fig. B.2. A figure of BJH adsorption indicate the ZSM-5 sample contains different pores.
Fig.B.3. Figure displays a TEM scan of ZSM-5 sample used in our NMRD analyses. The white circles indicate pinholes.
Appendix C Water proton $R_1$-NMRD relaxation model

Based on [1.17], the water proton $R_1$-NMRD relaxation rate $R_1$ can be expressed in term of field-independent term $\alpha$ and spectral densities which describe intra-and inter molecular dipole-dipole couplings.

$$R_1(\omega_0) = \alpha + \sum_{\mu} N_{\mu} \beta_{\mu,\text{intra}} F_{\text{intra}}(\omega_0 \tau_{\epsilon,\mu}) + \sum_{\mu} N_{\mu} \beta_{\mu,\text{inter}} F_{\text{inter}}(\omega_0 \tau_{\epsilon,\mu})$$  \hspace{1cm} (c.1)

Here $F(x)(...)\text{ terms (}x\text{ can be intra- and inter-) are dispersion functions:}$

$$F_{\text{intra}}(\omega_0 \tau_{\epsilon,\mu}) = \tau_{\epsilon,\mu} \left\{ \frac{0.2}{1 + \omega_0^2 \tau_{\epsilon,\mu}^2} + \frac{0.8}{1 + 4\omega_0^2 \tau_{\epsilon,\mu}^2} \right\}$$  \hspace{1cm} (c.2)

$$F_{\text{inter}}(\omega_0 \tau_{\epsilon,\mu}) = \tau_{\epsilon,\mu} \left\{ \frac{0.3}{1 + \omega_0^2 \tau_{\epsilon,\mu}^2} + \frac{0.6}{1 + 4\omega_0^2 \tau_{\epsilon,\mu}^2} \right\}$$  \hspace{1cm} (c.3)

The field-independent term $\alpha$ is interpreted as follow:

$$\alpha = R_{\text{bulk}} - \frac{N_{\alpha}}{N_T} R_{\text{bulk}} - \frac{N_{\beta}}{N_T} R_{\text{bulk}} + \frac{N_{\alpha}}{N_T} R_{\alpha}$$  \hspace{1cm} (c.4)

$R_{\text{bulk}}$ stands for the spin-lattice relaxation rate of the bulk which also field-independent as they both in extreme narrowing limit; $R_{\alpha}$ is determined as 0.2 s$^{-1}$; $N_{\alpha}$, $N_{\beta}$, and $N_T$ are number of water molecules for $\alpha$ waters, $\beta$ waters, and in total.

The dispersion amplitude parameters in equation (c.1) are given by:

$$\beta_{\mu,\text{intra}} = \frac{1}{N_T} \left( \frac{3}{2} \right) (D^\text{intra}_{\mu} A^\text{intra}_{\mu})^2$$  \hspace{1cm} (c.5)

$$\beta_{\mu,\text{inter}} = \frac{1}{N_T} \sum_{l} \frac{k}{2} \left[ (D^\text{inter}_{\mu l} A^\text{inter}_{\mu l})^2 + (D^\text{inter}_{\mu 2l} A^\text{inter}_{\mu 2l})^2 \right]$$  \hspace{1cm} (c.6)
\[ D^\text{intra}_h = \frac{\mu_0 h \gamma^2_h}{r^3_{h,j}}, \quad D^\text{inter}_h = \frac{\mu_0 h \gamma^2_h}{r^3_{h,j}} \]

Here D stands for dipolar coupling constant and A is the generalized orientational order parameter, they both associated with intra- and intermolecular dipole couplings; and k=3/2 for “like spins”, k=1 for “unlike spins”.